Sequencing and phylogenetic analysis of *Entamoeba histolytica* in cattle in Al-Diwaniyah governorate, Iraq

T.I. Jawad¹, H.H. Jawad² and M.A. Alfatlawi²

¹Technical Institute in Al-Diwaniyah, ²Department of Veterinary Microbiology, College of Veterinary Medicine, University of Al-Qadisiyah, Al-Diwaniyah, Iraq

**Abstract**

This study is carried out to evaluate the occurrence of *Entamoeba histolytica* in cattle in Al-Diwaniyah Governorate, Iraq. Fifty fecal samples were collected from cattle in different regions of the province. Microscopy and polymerase chain reaction (PCR) were used to examine the samples. Four-PCR-product-dependent gene-sequencing was conducted, besides the microscopy, targeting the small subunit rRNA (SSU rRNA) gene of the parasite. The outcomes of the microscopy demonstrated the presence of the parasitic cyst and trophozoite in 66%, (33/50) of the fecal samples of the examined cattle. The results of the PCR showed that the genus level of *Entamoeba* was positive in 72%, (36/50) of the fecal samples. The sequencing reported the existence of four closely similar isolates to isolates registered in fecal samples of cattle in Baghdad City, Iraq. The study concludes that *Entamoeba histolytica* is currently present in the cattle tested in the current governorate, and the zoonotic disease could strongly be induced in people with contact with the animals testing positive.

Keywords: Amoebic dysentery

**Correspondence:**

M.A. Alfatlawi

monyerr.abd@qu.edu.iq

DOI: [10.3389/ijvs.2023.137074.2635](http://doi.org/10.3389/ijvs.2023.137074.2635) ©Authors, 2023, College of Veterinary Medicine, University of Mosul. This is an open access article under the CC BY 4.0 license (http://creativecommons.org/licenses/by/4.0/).
body or by stomach secretions if consumed, cysts can persist for days to weeks in such environments. Trophozoites are able to attach to and destroy the colonic epithelium before blood-stream, spreading to far-off locations including the peritoneum, liver, lung, or brain via the portal vein system (15-17).

This study is carried out to evaluate the occurrence of *Entamoeba histolytica* in cattle in Al-Diwaniyah Governorate, Iraq.

**Materials and methods**

**Samples**

Fifty fecal samples were collected from cattle in different areas of Al-Diwaniyah Governorate, Iraq. The samples, which were collected in May-October, 2022, were capped in containers that were cold-transported to the Parasitology Laboratory, College of Veterinary Medicine, University of Al-Qadisiyah, Al-Diwaniyah Governorate, Iraq.

**PCR and DNA Sequencing**

Each fecal sample (200 mg) was washed in 1ml-sterile Phosphate Buffer saline pH 7.2, 5mins-14,000 × g-centrifuged, according to the instructions of QIAGEN kit (QIAGEN, Hilden, Germany), then, DNA was extracted. The primers employed in the current study were F: TGGACTTCAGGGGGAGTATG and R: TCAATCTCGGTACACCACTCA for a product of 545bp (18). DNA polymerase at 0.2µl 5U/µl, DNA at 2µl 100ng, each primer at 0.6µl 40µM, and 16.5µl PCR-water were used for the PCR reaction component. At the conditions, 94°C-Denaturation, 40 cycles of (92°C-60s denaturation, 47°C-60s annealing, and 72°C-90s-extension), and 72°C-7mins final extension were employed for the thermocycler. The PCR products were purified and sent to Macrogen (Korea) to perform partial gene sequencing. The phylogenetic tree was generated using MEGA X, and similar world sequences were detected using NCBI websites.

**Results**

The outcomes of the microscopy demonstrated the presence of the parasitic cyst and trophozoite in 66% (33/50) of the fecal samples from the tested cattle (Figure 1). The results of the PCR showed that the genus level of *Entamoeba* was positive in 72% (36/50) of fecal samples (Figure 2). The sequencing reported the existence of four closely similar isolates to isolates registered in Baghdad City, Iraq, (MW426065 and MW426066) (Figure 3).

**Discussion**

*Entamoeba histolytica* is commonly known as highly infectious parasite that causes a severe diarrheal disease in humans; however, some reports noted that this protozoan could cause a disease in animals (19). The current study...
demonstrated, via the use of microscopy, the species identification of the parasite; however, it is said that microscopy can often be employed to diagnose protozoa in stool samples, but to the genus level. In disagreement with the current result, researchers suggested that this approach is unable to distinguish *E. histolytica* from other non-pathogenic species like *E. dispar*, which has physical similarities with it (20). As a result, the WHO urged the creation and use of novel techniques for a precise diagnosis of *E. histolytica* infections (20), including the PCR, which can be a better choice for the significant detection of the protozoan.

The results of the current study ensured that the PCR is highly effective in the detection of *E. histolytica*. Hence, utilizing PCR is another option for separating the species. Compared to microscopy, this method has high sensitivity and specificity. A study by López-López et al. (20) used a piece of the adh12 gene to identify and to differentiate between *E. histolytica* and *E. dispar*. They found that this gene showed five variations in single nucleotide between the two species. *E. histolytica* and *E. dispar* were successfully detected using a variety of techniques, and real-time PCR. Unfortunately, the expensive price restricts their application in developing countries, and some of them also, provides falsely negative findings (21-37). Moreover, the SSu rRNA gene based PCR was highly effective in the current detection, and this agrees with López-López et al. (20).

According to the results of the microscopic study and PCR tests by Aryal et al. (38), it was revealed that 80% of the wild water buffaloes had *E. bovis* infection. Also, a research conducted in China showed that *E. bovis* infection affected 100% of cattle and more than 90% of yak and some small ruminants (39). In a Ugandan investigation, *E. bovis* infection was detected in 60% of goats and 80% of cattle (40).

**Conclusion**

The study suggested that *Entamoeba histolytica* is currently present in the cattle tested in Al-Diwaniyah Governorate, and that zoonotic infection could strongly be induced in people in contact with animals testing positive.

**Acknowledgments**

The authors thank Professor Jabbar Ahmed Alssady, Dean of College of Veterinary Medicine, University of Al-Qadisiyah, Iraq, for technical assistance.

**Conflict of interests**

The authors have not received any funding or benefits from industry, agency of financing, or elsewhere to conduct this study.

**References**


